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Metazoan parasites of *Geophagus brasiliensis* (Perciformes: Cichlidae) in Patos lagoon, extreme south of Brazil

Metazoários parasitos de *Geophagus brasiliensis* (Perciformes: Cichlidae) da Lagoa dos Patos, extremo sul do Brasil

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Abstract

This study has evaluated the parasitic fauna of 79 pearl cichlids (*Geophagus brasiliensis*) from the estuary of Patos Lagoon (31° 57’ S and 52° 06’ W), Rio Grande do Sul, Brazil, during the months of May and June in 2011 and 2012. All the hosts analyzed were infected with at least one species of parasite. A total of eleven metazoa were identified in 459 specimens collected. The trematode *Austrodiplostomum compactum* (34.2%) and ergasilids *Ergasilus lizae* (32.9%) and *Gauchergasilus lizae* (32.9%) were the most prevalent species. The trematodes *Thometrema overstreeti* and *Posthodiplostomum* sp. had significantly higher prevalence in fish longer than 20 cm. The sex of the host had no effect on parasite prevalence and abundance. Pearl cichlids are registered as a new host for the trematodes *Lobatostoma* sp., *Homalometron pseudopallidum* and *Thometrema overstreeti*, for the ergasilids *Ergasilus lizae* and *Gauchergasilus euripedesi* and for the argulid *Argulus spinolosus*. The crustacean *E. lizae* is recorded in Rio Grande do Sul for the first time.

Keywords: Helminths, Crustacea, Hirudinea, Cichlidae, parasitological indices.

Resumo

Este estudo avaliou a fauna parasitária de 79 *Geophagus brasiliensis* (acarás) proveniente do Estuário da Lagoa dos Patos (31°57’S e 52°06’W), Rio Grande do Sul, no período de Maio e Junho de 2011 a 2012. Todos os hospedeiros analisados estavam infectados com pelo menos uma espécie de parasito. Um total de onze metazoários foi identificado em 459 espécimes coletados. O trematoda *Austrodiplostomum compactum* (34,2%) e os ergasilídeos *Ergasilus lizae* (32,9%) e *Gauchergasilus lizae* (32,9%) foram às espécies mais prevalentes. Os trematodeos *Thometrema overstreeti* e *Posthodiplostomum* sp. apresentaram uma prevalência significativamente maior nos peixes acima de 20 cm. O gênero sexual dos hospedeiros não apresentou influência sobre a prevalência e a abundância parasitária. *Geophagus brasiliensis* é registrado como um novo hospedeiro para os trematódeos *Lobatostoma* sp., *Homalometron pseudopallidum* e *Thometrema overstreeti,* para os ergasilídeos *Ergasilus lizae* e *Gauchergasilus euripedesi* e para o argulídeo *Argulus spinolosus.* O crustáceo *E. lizae* é registrado pela primeira vez no Rio Grande do Sul.

Palavras-chave: Helmintos, Crustacea, Hirudinea, Cichlidae, índices parasitológicos.

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Introduction

The Patos Lagoon, located in the southernmost area of Brazil, is 265 km long and has 10.000 km^2 of surface area. It is thus one of the largest coastal lagoons in the world. The estuary of the Patos Lagoon encompasses over 900 km^2 in the southernmost area of the lagoon (BONILHA & ASMUS, 1994).

Although there is great diversity of fish in the estuary of the Patos Lagoon, there are few studies on the parasitic fauna of these fish. In the area of the estuary, parasitological studies have been conducted on *Micropogonias furnieri* (Desmarest, 1823) (VELLOSO & PEREIRA, 2010), *Paralichthys orbignyanus* (Valenciennes, 1839) (VELLOSO et al., 2005) and *Mugil platanus* (Günther, 1880) (EIRAS et al., 2007).

Parasite diversity in host fish depends directly on the degree of diversity of the habitat (D'AMELIO & GERASI 1997; GELNAR et al., 1997). Thus, many studies have been conducted regarding the use of fish parasites as environmental bioindicators (SURES, 2003, 2004; MARCOGLIESE, 2005; NACHEV et al., 2010; VIDAL-MARTÍNEZ et al., 2010; KHAN, 2011).

In aquaculture, situations that lead to the rupture of the host-parasite-environment balance can trigger disease outbreaks. In addition to the consequences of parasitism itself, parasitized fish are more susceptible to secondary infections by bacteria and fungi. Thus, their zootechnical and reproductive performances are compromised and they transmit pathogenic agents to the farming environment, thereby leading to great economic losses (MARTINS, 1998; LIMA & LEITE, 2006).

In relation to public health, parasitic zoonoses transmitted through fish consumption have increasingly drawn the attention of researchers and sanitation authorities worldwide, due to consumption of raw or insufficiently cooked fish (BOUREE et al., 1995; LUQUE, 2004).

Geophagus brasiliensis (Quoy & Gaimard 1824), known as the *acará*, *caraúna*, *acará-ferreira* or pearl cichlid, occurs in rivers, streams and ponds in South and Central America. This species is used in recreational fishing in fee-fishing ponds because they can easily reproduce and also in the aquarium trade due to their attractive coloration (BIZERRIL & PRIMO, 2001). Studies conducted with the aim of using *G. brasiliensis* in aquaculture in southern Brazil have shown that it presents good development in cold waters, thus highlighting the importance of using a native fish species as an alternative for diversifying species in a farming system (AMARAL et al., 2011).

During the initial development stages of *G. brasiliensis*, these fish are plankton-eaters (LAZZARO, 1991). Later on, they become omnivorous bottom-dwellers and present territorial behavior (BEATTY et al., 2013). However, this characteristic, as well as marked differences in behavior and size (length and weight) between males and females, is reflected in the composition and mean value of their food consumption. In addition, for *G. brasiliensis* in the Patos/Mirim lagoon system, gastropods are the most important component of their diet (40.64% among adult males and 35.55% among adult females), with important variations in diet relating to the stage of maturity of this host (BASTOS et al., 2011). In Brazil, studies on helminths parasitizing *G. brasiliensis* have been reported in the Rio de Janeiro (PARAGUASSU et al., 2005; AZEVEDO et al., 2006; CARVALHO et al., 2010) Parana (BELLAY et al., 2012). Because of the importance of parasitic diseases in fish with regard to public health and, and as environmental bioindicators. The present study was proposed with the objective of identifying the parasitic fauna and its infection levels in *G. brasiliensis* from the southernmost area of Brazil.

Material and Methods

A total of 79 specimens of *G. brasiliensis* (30 females, 43 males and six unidentified), were collected from the estuary of the Patos Lagoon by local fishermen using nets and casting nets, in the months of May and June in 2011 and 2012. After the fish had been caught, they were placed in a polystyrene box with ice and were transported to the Parasitology Laboratory of the Federal University of Pelotas. Firstly, the fish were measured (total length [TL] and standard length [SL]) and weighed, and a thorough external evaluation was performed in order to collect ectoparasites. Next, the fish were necropsied. Their organs were removed and placed individually in Petri dishes for helminth inspection.

Collection, fixation and quantification of parasites were performed in accordance with Eiras et al. (2006). Prevalence, mean intensity and mean abundance values were calculated in accordance with Bush et al. (1997). The influence of the sex of the host on the abundance and prevalence of parasitic infections was analyzed through the Bootstrap-t test and chi-square test respectively, and $p \leq 0.05$ was taken to be significant. Pearson's correlation coefficient was used to determine possible correlations between the total length of the host and the prevalence of parasitic infection. The samples from the hosts were separated into nine class intervals of amplitude 2 cm, with previous angular transformation of the prevalence data (ZAR, 1999). Spearman's rank correlation coefficient ("rs") was used for determining the correlation between the standard length and abundance of parasite species (ZAR, 1999).

Results

The biometric parameters of *Geophagus brasiliensis* evaluated are described in Table 1.

All the hosts were infected by at least one species of metazoan parasite. A total of 459 specimens of metazoan parasites were found, and eleven genera and eight species were identified (Table 2). Digeneans were the most diverse group, represented by six species. Metacercariae of *Austrodiplostomum compactum* (Lutz, 1928) were found in the eyes of the hosts (Figure 1a, b) and were the most prevalent trematode (34.2%), followed by *Lobatostoma* sp. (28.76%), which was found in the intestines. Among the ectoparasites, the ergasilids *Ergasilus lizae* (Kroyer, 1863), *Gauchergasilus euripedesi* (Montú, 1980) (32.9%) and Glossiphinidae gen. sp (24.05%) predominated, parasitizing the gills.

Thometrema overstreeti (Brooks et al., 1979) (adults in the intestines) and *Posthodiplostomum* sp. (metacercariae in the eyes and on the surface of the swim bladder) presented significantly higher prevalence in fish longer than 20 cm. The sex of the host

Table 1. Biometric parameters of 30 females and 43 males of Geophagus brasiliensis during May and June 2011 and 2012, in Patos Lagoon, Rio Grande do Sul, Brazil.

	Geophagus brasilienses	**Female**	**Male**
Species examined	73	30	43
Mean weight	270.28 g	254.04 g	301.44 g
Mean total lenght	22.04 cm (±3.13)	21.53 cm	23.04 cm
Mean Standard length	17.52 cm (±3.27)	17.33 cm	18.51 cm

did not present any influence on the prevalence or abundance of the metazoan parasites found in *G. brasiliensis.*

Discussion

Eleven parasite taxa were identified, with greatest representation of trematodes, as also observed in studies conducted in Rio de Janeiro on this same host (AZEVEDO et al., 2006; CARVALHO et al., 2010). This is probably due to the omnivorous-opportunistic habits of these fish and because they cohabit with a wide diversity of

Table 2. Parasitological indexes of metazoans from *Geophagus brasiliensis* (n = 79) from Patos Lagoon, Rio Grande do Sul, Brazil.

Parasite	P(%)	MA ±SD	MI±SD	Range	Site of infection
Trematoda					
Austrodiplostomum compactum (metacercariae)	34.20	1.49 ± 3.43	4.37 ± 4.73	1-118	Eyes
Clinostomum marginatum (metacercariae)	13.90	0.87 ± 3.39	6.27 ± 7.24	1-69	
Homalometron pseudopallidum	2.74	0.12 ± 0.8	5 ± 1.41	1-10	Intestine
Lobatostoma sp.	28.76	0.55 ± 1.35	2.09 ± 1.94	1-44	Intestine
Posthodiplostomum sp. (metacercariae)	12.65	0.25 ± 0.72	2 ± 0.81	1-20	Eyes and on the surface of the swim bladder
Thometrema overstreeti	16.45	0.16 ± 0.72	2.6 ± 1.51	1-13	Intestine
Nematoda					
Contracaecum sp. (larvae)	2.74	0.05 ± 0.31	2 ± 0	1-4	Intestine
Crustacea					
Branchiura					
Argulus spinulosus	7.59	0.1 ± 0.37	1.33 ± 0.51	1-8	Gills
Copepoda					
Ergasilus lizae	32.91	1.05 ± 1.9	3.19 ± 2.05	1-83	Gills
Gauchergasilus euripedesi	32.91	0.79 ± 1.31	2.42 ± 1.17	1-63	Gills
Hirudinea					
Glossiphinidae gen. sp.	24.05	0.24 ± 0.66	1.46 ± 0.96	1-19	Gills

P (Prevalence), MA (Mean Abundance) and MI (Mean Intensity).

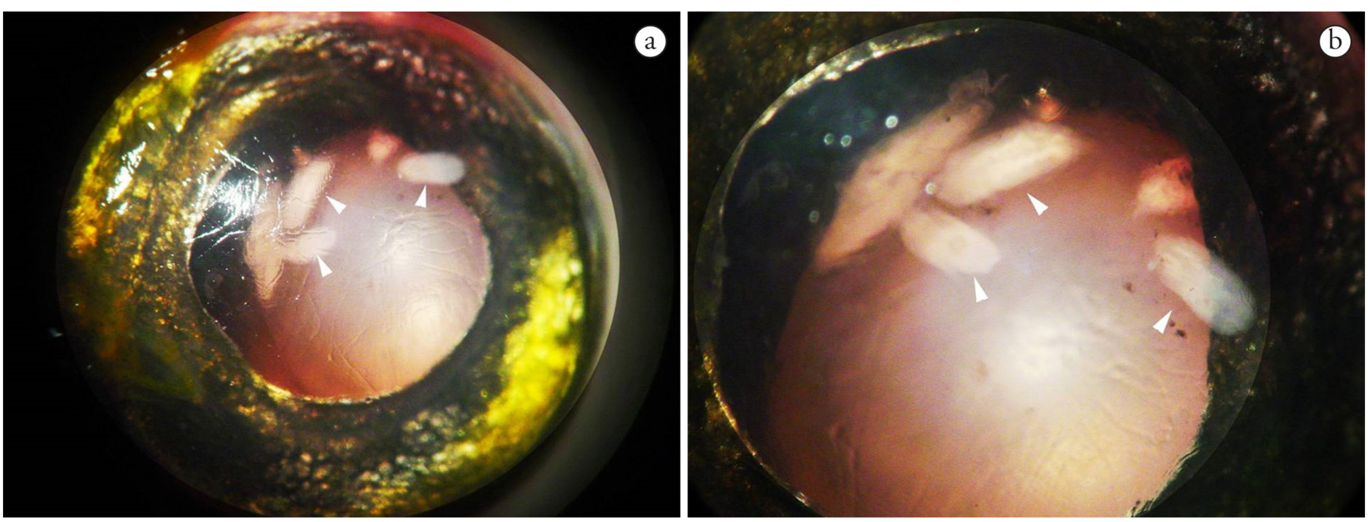

Figure 1. (a) The eye of the *Geophagus brasiliensis* infected by metacercariae of *Austrodiplostomum compactum*. (b) Detail showing the metacercariae of *A. compactum* in the eye of the *G. brasiliensis.*

intermediate hosts, since many of these trematodes use two or more hosts to complete their biological cycle (EIRAS, 1994). In addition, *G. brasiliensis* is a benthic species, and this habit favors contact with mollusks, which act as intermediate hosts for trematodes (MARCOGLIESE, 2002).

Metacercariae of *A. compactum* presented the highest prevalence (34.2%) among the parasites reported. Santos et al. (2012) analyzed *Geophagus surinamensis* (Bloch, 1791) and observed higher prevalence (46.1%), which was associated with water temperatures that ranged from 21.4 to 29°C. Previous studies also suggested that the biology of this parasite depends on high temperatures (MARTINS et al., 2002; HAKALAHTI et al., 2006). In the present study, although the prevalence of this trematode was 34.2%, all the sampling was performed between May and June, when the mean environmental temperatures are lower. Cercariae are probably released from gastropods during periods of higher temperatures, thus resulting in accumulation of the parasites in colonization processes that occur during these higher-temperature periods. The fact that gastropod mollusks comprise the main component of the diet of *G. brasiliensis* in the Patos/Mirim lagoon system may have contributed to these values (BASTOS et al., 2011). Moreover, the same authors showed that significant variation in the diet occurs, according to the host's maturity stage. Other facts may have favored transmission, such as strategies of releasing cercariae close to the intermediate host and the lentic condition of the environment (SANTOS et al., 2012). Regarding the infection site of *A. compactum* in the host, this has been recorded on the surface of the swim bladder (CARVALHO et al., 2012). However in the present study, in which total necropsies were performed, metacercariae of *A. compactum* were only reported in the eyes of *G. brasiliensis,* which makes it possible to accept the hypothesis postulated by Eiras (1994), i.e. that different species of Displostomidae can present specificity for infection sites in the host.

In the present study, *Lobatostoma* sp. is reported for the first time in *G. brasiliensis*. *Lobatostoma jungwirthi* was described in *Geophagus brachyurus* (Cope, 1894) in the Sinos River, São Leopoldo, RS, Brazil, by Kritscher (1974). This same species was recorded in the freshwater mollusk *Heleobia castellanosae* (Gaillard, 1974) in Buenos Aires, and this is the only species that parasites freshwater fish. Thus, it can be suggested that the *Lobatostoma* sp. reported here may be the same species described by Zylber & Nunez (1999). According to these authors, the lifecycle of *L. jungwirthi* is heteroxenous and very similar to what was described by Rohde & Sandland (1973), Rohde (1994) for *Lobatostoma manteri*, because both species need a vertebrate host to complete their lifecycles. Rohde & Sandland (1973) observed that in mollusks, the larvae hatch in the stomach and, depending on the species, remain in this organ or migrate to the digestive glands, where they grow until the pre-adult stage, presenting all the adult characteristics, including one testicle and ovary. However, they do not eliminate eggs, and fish are infected through ingestion of these mollusks. Thus, the transmission of this trematode must be associated with ingestion of gastropod mollusks, which are one of the main components of the diet of *G. brasiliensis* in the Patos/Mirim lagoon system (BASTOS et al., 2011).

Black spot disease, which occurs frequently in many species of fish, is caused by a variety of parasite species but predominantly by metacercariae of *Posthodiplostomum* sp. In the present study, metacercariae of *Posthodiplostomum* sp. were found in the eyes and on the surface of the swim bladder. Azevedo et al. (2006) reported *P. macrocotyle* (Dubois, 1937) in *G. brasiliensis*, in their eyes, mouth cavity, stomach and gonads.

The presence of metacercariae of *Clinostomum* sp. on the external surface of the fish causes black spot or yellow spot disease, which can lead to economic losses because it hampers commercialization of the fish, can cause death among young hosts and also makes them more susceptible to attack by predators. Metacercariae of *Clinostomum marginatum* (Rudolphi, 1819) were found in the fins of *G. brasiliensis*. There are previous records of this parasite in other species of cichlids such as *Cichla ocellaris* (Bloch & Schneider, 1801) and *Crenicichla* sp., in their gills, skin and fins (THATCHER, 1981). *Clinostomum* sp. was also reported by Paraguassú et al. (2005) in the fins of *G. brasiliensis* with lower prevalence (3%).

This was the first report of *Thometrema overstreeti* in *G. brasiliensis*. It has already been reported in *Pimelodus maculatus* (Lacépéde, 1803) in southern Brazil (KOHN et al., 1990). The latter is also an opportunistic omnivorous bottom-dwelling fish, which allows completion of the parasite's cycle.

Another trematode found in *G. brasiliensis* in this study was *Homalometron pseudopallidum* (Martorelli, 1986), which has been reported in over 20 species of fish, especially marine fish, but with few records in freshwater fish. Thus, this was the first report of *H. pseudopallidum* in *G. brasiliensis.* This species was reported in *Gymnogeophagus australis* (Eigenmann, 1907) in Argentina (KOHN et al., 2007). Both of these hosts belong to the same family; they share the same feeding habit and co-occur in rivers and ponds in southern Brazil (REIS & MALABARBA, 1987).

The only nematode detected was *Contracaecum* sp. (Anisakidae), which was found in the larval stage. Species of *Contracaecum* sp. are of public health importance because they are responsible for emerging diseases such as anisakiasis, which affects humans after consuming raw or poorly cooked fish dishes, such as sushi, sashimi and ceviche (MINETA et al., 2006; FELIZARDO et al., 2009). No cases of this have been reported in humans in Brazil, although there have been reports of occurrences of larvae of anisakids in various fish (BARROS et al., 2006, 2007; SAAD & LUQUE, 2009; KNOFF et al., 2013; MATTOS et al., 2014). Reports of larvae of *Contracaecum* sp. sampled in *G. brasiliensis* in Brazil have presented higher rates, as reported by Paraguassú et al. (2005) (14%) and Bellay et al. (2012) (40.62%) in reservoirs in the states of Rio de Janeiro and Paraná, respectively. In turn, in studies conducted in the Guandu River in the state of Rio de Janeiro, Azevedo et al. (2006) and Carvalho et al. (2010) both observed a prevalence of 6%, i.e. close to what was found in the present study. These differences may be associated with the time at which sampling was performed, as well as with the availability of hosts used by *G. brasiliensis* in its food chain. Although larvae of *Contracaecum* sp. were only found in the intestinal lumen of *G. brasiliensis* at low parasitological levels, the zoonotic risk is not eliminated, given that there is a possibility of migration of these larvae to the muscle tissue of the host, both in the living fish and after capture, especially because of the length of time for which the fish remain in the boat and/or in the fish warehouse, without being eviscerated, as well as through the use of viscera in typical dishes (BOUREE et al., 1995; LYMBERY & CHEACH, 2007).

Regarding ectoparasites, the argulid *Argulus spinulosus* (Silva, 1980), ergasilids *E. lizae* and *G. euripedesi* and leeches Glossiiphonidae gen. sp. (Hirudinea) were identified.

Argulus spinolosus has already been reported in another cichlid, *Oreochromis niloticus* (Linnaeus, 1758), in traditional fish-farming tanks in Santa Catarina, with prevalence of 33% (GHIRALDELLI et al., 2006), i.e. greater than what was found in *G. brasiliensis* in the present study (7.59%). During the reproduction period of argulids, females abandon the host and search for solid substrates to lay their eggs. Infecting larvae are able to swim and infect new fish, thus continuing the lifecycle (EIRAS, 1994). Thus, the confined fish-farming environment can facilitate the lifecycle of argulids (GOMES & MALTA, 2002).

The ergasilid *G. euripedesi* is widely distributed in estuarine waters, from the state of Rio Grande do Sul to Sergipe (MONTÚ & BOXSHALL, 2002). This ergasilid has already been reported in the gills of *M. furnieri*, which were also sampled in the same estuary of Patos Lagoon (VELLOSO & PEREIRA, 2010).

Ergasilus lizae has already been reported parasitizing the gill filaments of *Mugil curema* (Valenciennes, 1836) (CAVALCANTI et al., 2011) and *Mugil planatus* (Günther, 1880) (LUQUE & TAVARES, 2007) in the state of Rio Grande do Norte. For the state of Rio Grande do Sul, this is a new record of occurrence. When fixed in the gills, they cause partial or total occlusion of the blood vessels of the lamellae, as well as hyperplasia and increased mucus, thus causing reduction of the respiratory ability of the host and facilitating occurrences of secondary infections (EIRAS, 1994).

Glossiphoniidae is composed of species that occur in freshwater environments on all continents except Antarctica. In the gills of *G. brasiliensis*, in the present study, the prevalence of parasitism due to leeches Glossiphoniidae gen. sp. (13.7%) was similar (10%) to what was observed in Guandu River in Rio de Janeiro (AZEVEDO et al., 2006). However, this was lower that the values observed in the Lajes reservoir in the same state (74%) (PARAGUASSÚ et al., 2005). According to Eiras (1994), the most important consequence of this parasitic disease is that leeches have the ability to transmit protozoa and other pathogens such as *Trypanosoma* spp. and haemogregarines, to fish.

The helminths *T. overstreeti* and *Posthodiplostomum* sp. presented a correlation between body length and prevalence, thus indicating that larger fish present higher chances of becoming infected by parasites. This corroborates the affirmation of Takemoto & Pavanelli (2000) that the greater surface area and viability of space in large fish and the larger amount of food that they ingest can favor higher levels of parasitism.

Conclusions

Geophagus brasiliensis is a new host for the trematodes *Lobatostoma* sp., *Homalometron pseudopallidum* and *Thometrema overstreeti,* as well as for the ergasilids *Ergasilus lizae* and *Gauchergasilus euripedesi* and for the argulid *Argulus spinolosus. Ergalisus lizae* is reported for the first time in Rio Grande do Sul. The sex of the host does not represent a determining factor for parasitism. The trematodes *Thometrema overstreeti* and *Posthodiplostomum* sp. presented significantly higher prevalence in fish longer than 20 cm.

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